MOLYBDENUM IN THE NUTRITION OF THE RABBIT AND RAT

By

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

June, 1952

UNIVERSITY OF FLORIDA
3 1262 08552 2786

ACKNOWLEDGEMENTS

In the planning of this research, in conducting the experiments and in preparation of this dissertation, acknowledgement is made of the help and counsel of many individuals. The author wishes to express sincere appreciation to all those who have contributed to the completion of this dissertation.

Dr. George K. Davis has made available necessary facilities for this research and as chairman of the supervisory committee has guided the research program.

Dr. R. B. Becker, Dr. T. W. Stearns, Dr. Ray L. Shirley, Dr. H. D. Wallace, Dr. A. F. Novak and Dr. R. L. Emerson served as members of the special supervisory committee.

Mr. Sam L. Hansard has given material aid and advice in the experiment involving the use of radioactive isotopes.

Mr. J. D. Burke made blood volume determinations on the experimental rabbits.

Dr. John F. Feaster has given personal assistance and cooperation.

Mr. John T. McCall and other members of the staff of the Nutrition Laboratory have aided in the laboratory analyses.

Dr. Max A. Jeter aided in the planning of some of the experiments and other graduate students have assisted in other phases of the research.

Mr. Juan Sola, Mr. Richard Sparrow and Mr. Max Garhart have contributed by attending the experimental animals.

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INTRODUCTION

Although molybdenum is one of the scarcer mineral elements, its compounds are widely distributed in nature. It occurs in subterranean deposits and in small but varying amounts in the surface layers of soil where it is available to plants. It is present in plant and animal tissues. Its wide occurrence in plants and animals has stimulated much interest in evaluating its function in these forms of life. Thus it is established that molybdenum is essential to the growth of certain microorganisms and many higher plants. It is not known to be a dietary requirements for any animal species; rather, its presence is harmful under certain conditions.

Animal species vary in their tolerance to molybdenum in the diet, ruminants being less tolerant than are non-ruminants. The action of molybdenum in the animal body whereby the toxicity is produced is not known.

There are areas of the world where the presence of molybdenum in the soil and in the plants makes grazing hazardous or impossible. While these areas are rather restricted, a potential problem exists which cannot be limited to areas now affected. Since many plants respond to the presence of molybdenum and reflect this to some extent in their tissues, the practice of molybdenum fertilization creates a problem which may be extended to other areas.

This study has been designed to extend observations of the effects of dietary molybdenum to the rabbit and expand studies with

the rat. The herbivorous dietary habit of the rabbit and the size of the animal make it possible to study a herbivorous species under laboratory conditions. The difference in the response of ruminant and non-ruminant animals toward molybdenum makes it desirable to establish the relative position of this herbivorous, but non-ruminant species, with regard to its tolerance for dietary molybdenum.

Molybdenum in Plant and Animal Nutrition

The wide distribution of molybdenum in the plant and animal kingdom has been observed by several investigators. In 1931 ter Meulen (59, 60) reported the presence of molybdenum in microorganisms, soil, wood, peat and in the blood, liver and spleen of domestic animals and man. Since that time it has been found in hens eggs (32, 56) and in human and cows milk (4, 33, 34).

The role of molybdenum in the nutrition of certain microorganisms was recognized before its significance in higher plants was established (10). In 1931, Bortels (11) reported that molybdenum is essential to biological nitrogen fixation. Since that time there have been many reports of investigations which have established the role of molybdenum as a stimulant to growth and nitrogen fixation in certain microorganisms (13, 19, 31, 47, 72, 73).

Some higher plants have shown favorable response to the presence of molybdenum in the soil. Arnon and Stout (5) in 1939 demonstrated the need for molybdenum in the nutrients of tomato seedlings and later its value for lettuce was established (16). Molybdenum is required by many plants (29, 46, 64, 77). Leguminous plants exhibit a greater

response to molybdenum than do non-legumes. In these plants it has been observed to increase growth or yield, to improve the color and stimulate nitrogen fixation (1, 2, 12, 48, 78).

Under certain soil and climatic conditions, plants accumulate excessive quantities of molybdenum which may become critical in relation to their use as pasture or hay crops. Some of these factors which may alter the accumulation of molybdenum have been established. A species difference has been established from the observations that leguminous plants concentrate more of the soil molybdenum than do non-legumes (8, 40, 52). An alkaline reaction of the soil favors an increase of the element in plant tissues whereas very little molybdenum is taken up under acid soil conditions (3, 8, 53). Young growing plants contain more molybdenum than mature plants on a dry matter basis (8, 37). The amount of the element in the soil is also a factor although its effect upon plant tissue accumulation is often masked by the form in which it is found and other soil conditions (53). Where these conditions exist, serious abnormalities in grazing cattle or sheep have been common. Such abnormalities have been reported from areas in California and Southern Florida in the United States and in England, Australia and New Zealand. While the conditions are not the same in all of these areas, the forage from these areas contains a relatively high amount of molybdenum.

Beath and associates (9) in 1935 observed certain abnormalities in cattle which were grazing pastures containing 89 ppm molybdenum. The first specific description of pathological conditions in cattle

which were attributed to excess molybdenum was made by Ferguson in 1938 (39). Since that time his observations have been confirmed and extended in the other areas of the world where cattle and sheep are similarly affected.

The external appearance of the plants gives no evidence of abnormality and only by analysis is the excess molybdenum detected. When plants from an affected area become mature or are made into hay, the toxicity is much reduced. It is possible that the green forage contains a form of molybdenum which is more toxic or that it contains another substance which renders the molybdenum more effective. When molybdate salts are fed to animals receiving dry hay and to those receiving green succulent feed, the toxicity is less severe or even absent in those receiving dry hay (37, 38, 39).

The toxic symptoms which have been observed in cattle and sheep are characterized by diarrhea, loss in weight, change in the color of the hair coat, anemia and eventual death unless removed from the affected area or treated (8, 17, 25). Horses grazing on the same areas which cause trouble in ruminant animals are not affected (17, 39).

Investigations concerning the effects of dietary molybdenum have not been limited to farm animals. Studies have been undertaken using laboratory animals, but these have been principally confined to experiments using rats (21, 36, 41, 42, 49, 63, 75). The results are in general agreement that rats are more tolerant of dietary molybdenum than ruminant animals; nevertheless, rats are subject to some toxic

effects of excess molybdenum. The manifestations of the toxic symptoms observed in rats do not parallel those in cattle and sheep. Severe diarrhea and anemia which are common symptoms in ruminants have not been consistently produced in rats.

Molybdenum as molybdenum trioxide, calcium molybdate, and ammonium molybdate supplying 1200 to 6000 milligrams of molybdenum per kilogram was fatal to guinea pigs and rats (36). An equivalent amount of molybdenite was not fatal.

Other abnormalities which have been more recently attributed to molybdenum are interferences with normal reproduction. Jeter (49) in 1951 found that a high percentage of male rats which were fed 80 and 140 ppm molybdenum in the diet were sterile. In female rats fed the same rations there was an irregular estrus cycle. Thomas and Moss (76) have observed infertility and a lack of libido in young dairy bulls. In both of these species, the male infertility was accompanied by damage to the germinal epithelium and interstitial cells of the testes.

Molybdenum and Copper Interrelationships in the Animal Organism

One of the most significant discoveries with regard to molybdenum toxicity is the therapeutic action of copper. When adequate amounts of copper are present in the dietary (the quantity necessary depends upon the molybdenum present) the symptoms of molybdenum toxicity seldom develop. Furthermore, toxic symptoms which may be present can be corrected with copper supplementation. This action of copper has been

demonstrated in practically all species for which a toxicity has been reported (25, 37, 38, 63). When there is an excess of molybdenum and a deficiency of copper in the soil which is normally reflected in the plant, the adverse effects upon animals grazing these plants can be corrected by copper fertilization (25).

The abnormalities caused by copper deficiency and molybdenum excess are such that it is frequently difficult to distinguish between the two (28, 74). Because of the therapeutic effect of copper, it might be expected that excess molybdenum should deplete the body of its stores of copper and create a copper deficiency. Such a decrease has not been consistently observed. Dick and Bull (30) reported a decrease in the copper content of the liver of ewes which were fed copper and molybdenum, but others (51, 58) have observed an increase in liver copper under similar conditions. When the copper intake is below that necessary to insure positive copper balance, molybdenum will reduce the copper stores more rapidly. However, when adequate copper is provided, molybdenum causes an increase in the copper store but at the same time may precipitate the symptoms of copper deficiency. Molybdenum apparently tends either to fix the copper in a form which is not available to the tissues or to antagonize by hindrance the copper containing enzymes (58).

The physiological action of molybdenum and the mechanism through which copper exerts its therapeutic effect are unknown. Based upon some evidence, several different theories have been proposed which are designed to explain these functions. McGowan and associates (55)

believe that the activity of bacteria in the gastrointestinal tract is controlled by catechols and that molybdenum renders the catechols ineffective by formation of complexes. The uncontrolled activity of the bacteria thus causes the severe diarrhea. The therapeutic effect of copper is thought to be due to its toxic action upon bacteria. This theory is not generally accepted in light of present knowledge. Wisconsin workers (63) have suggested that the action of molybdenum may be due to the formation of a copper molybdate complex which is poorly absorbed. The effect of molybdates upon certain enzymes *in vitro* and the interaction of copper and molybdenum *in vivo* have suggested to others that molybdenum interferes with some enzyme systems, particularly the copper enzymes (7, 50). None of these theories has yet been supported by adequate evidence to explain the action of molybdenum.

Molybdenum and Phosphorus Metabolism

Among the symptoms of molybdenum toxicity which have been observed in cattle has been an abnormal skeletal development. This has been accompanied by other evidences of abnormal phosphorus metabolism. In areas of Florida where there is a deficiency of copper, and molybdenum is present, abnormal bone formation has resulted (26, 27). Specific symptoms have been stiffness, broken bones, enlarged joints, erosion of the joints and a condition resembling rickets in young animals and osteomalatia in older cattle. Thomas and Moss (76) have observed stiffness and bone involvement in young dairy bulls fed molybdenum. The plasma inorganic phosphorus and serum phosphatase were

normal, but there was erosion of some joints and union and fusion in one joint. The ash content of rib bones was normal. The stiffness and brittleness of bones which have been observed by others (17, 25, 26) also reflect abnormal bone development, possibly related in some manner to the metabolism of molybdenum.

Some of the effects of molybdenum upon absorption and excretion of phosphorus have been studied using radioactive phosphorus^{32}. When phosphorus and molybdenum were administered simultaneously to rats, the phosphorus uptake was increased. When molybdenum was fed previously there was the reverse effect upon phosphorus (21). The normal excretion of phosphorus by cattle where most of the phosphorus is excreted in the urine has been observed to change to a pathway where more is excreted in feces when the animals were given copper and molybdenum supplements (68). No similar effect could be observed in rats, although there was a change in the rate of excretion (69). These results further indicate some abnormality in phosphorus metabolism.

Other possible relationships between molybdenum and phosphorus metabolism have been shown through the action of molybdate ions upon phosphatases. Bossard (14, 15, 22) has reported an inhibitory effect of the molybdate ion upon certain phosphatases including a slight inhibitory effect upon calf bone phosphatase. Also observed was an increase in serum alkaline phosphatase and blood inorganic phosphorus in copper deficient cattle (26).

The literature concerning molybdenum and its relation to other elements in the animal organism has been reviewed by several authors.

The relation of certain minerals to animal health has been reviewed by Russell (65) and the relation of mineral elements to health of ruminants in Great Britain has been summarized by Green (43). The role of molybdenum and other minerals in the nutrition of animals and plants has been more recently reviewed by Marston (57).

EXPERIMENT I

MOLYBDENUM TOXICITY IN THE RABBIT

Some differences in species with relation to their tolerance for molybdenum have been observed. Limited studies with horses and swine have indicated a relatively high tolerance for these species and the numerous investigations with cattle and sheep show that these animals have a low tolerance for molybdenum. Because of the different effects of molybdenum upon ruminant and non-ruminant animals, it appeared desirable to determine whether or not the element was toxic to rabbits and to establish its relative toxicity in this species. Upon the initiation of this study there had been no reported investigations of the effect of molybdenum upon rabbits. Smith and Ellis (70) have described the symptoms of simple copper deficiency in the rabbit, but there is no evidence that excess molybdenum was involved in the abnormality.

The objective of this experiment was to describe the effects of molybdenum excess upon rabbits.

Experimental Procedure

Male and female Dutch rabbits of different ages were used for this experiment. They were maintained in metal cages with metal wire bottoms. The rabbitry was housed in an open barn with natural ventilation and without artificial heat.

The basal ration for all treatments was a commercial ration in pellet form (Staf-O-Life Rabbit Pellets, manufactured by Royal

Staf-O-Life Mills, Memphis, Tennessee) and had promoted satisfactory growth for rabbits in this laboratory. Feed was given ad libitum and all unconsumed feed was removed every other day or more often if it became dirty. Small quantities of fresh green feed were given each week. Fresh water was supplied from glass drinking bottles.

Molybdenum was added to the basal ration as sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in amounts calculated to provide 0.014, 0.05, 0.1, 0.2 and 0.4 per cent molybdenum. The basal ration contained 2.7 ppm molybdenum and 16.4 ppm copper by analysis using the thiocyanate-stannous chloride method (67) for molybdenum and the carbamate method (66) for copper. Molybdate salt was added to the pellets by spraying on an aqueous solution and allowing it to air dry. Analyses of composite samples made from the rations indicated that at the higher levels of molybdenum the amount incorporated was slightly less than the calculated amount.

Thirty-four weanling rabbits six to seven weeks of age were placed on the experimental rations and six mature rabbits were changed from the basal ration to three of the treatment levels of molybdenum. Observations were made daily for any development of toxic symptoms and the order of appearance of any abnormalities was recorded.

Results and Discussion

Gross abnormalities which were attributed to excess molybdenum developed in the rabbits fed 0.1 per cent or more molybdenum. Deaths resulted from the higher levels of molybdenum. A summary of the results is presented in Table 1. All of the young rabbits receiving 0.1 per

cent molybdenum developed some symptoms of toxicity although the condition became severe in only three of these rabbits. These three were treated with copper when it became apparent that they would not survive without treatment. No detrimental effects of 0.1 per cent molybdenum were observed in the two mature rabbits over a period of eight weeks. Five-hundredths per cent molybdenum in the ration caused no gross abnormalities in any of the animals. There was some evidence of retarded growth of the rabbits on this level of molybdenum in the early weeks after weaning. There was a slight loss of hair which soon returned to normal, but other symptoms of toxicity were absent. None of the rabbits receiving 0.014 per cent molybdenum developed abnormalities which could be attributed to molybdenum in the ration.

TABLE 1

SUMMARY OF MOLYBDENUM TOXICITY SYMPTOMS IN RABBITS

Treatment	No. of Rabbits	Deaths	Anemia	Alopecia and Dermatosis	Front leg Abnormality
0.4% Mo.	4	4	present	0*	1
0.2% Mo.	5	4	present	4	2
0.1% Mo.	7	**	present	5	2
0.05% Mo.	5	0	absent	0	0
0.014% Mo.	12	0	absent	0	0
Basal	7	0	absent	0	0

* Deaths occurred early and no alopecia observed.
** Severe toxic symptoms developed in three rabbits with deaths probable but copper therapy was initiated to prevent death.

The time of appearance of the abnormalities varied from about four weeks in the weanling rabbits to several months in the mature animals. Following the first appearances of the condition, the syndrome became progressively worse and death usually occurred within two weeks.

The syndrome which developed was characterized by a decrease in feed intake, a decrease in weight gains or actual weight loss, roughening of the hair coat with loss of hair beginning with complete denudation over small areas which generally spread to practically the entire body. The alopecia was accompanied by varying degrees of dermatosis with rough, dry, scaly skin. There was no evidence of skin infection or inflammation. Anemia became severe and the animals soon died unless treated. In some young rabbits an abnormality in the front legs was present.

The anemia, alopecia and dermatosis make the condition appear similar to the symptoms of copper deficiency in rabbits described by Smith et al (70). In contrast to the simple copper deficiency symptoms, no graying of the black hair in the rabbits on this experiment was observed. In Figure 1 are pictures of rabbits exhibiting typical alopecia.

Limited observations were made of the sex interest of male rabbits. Two males on high levels of molybdenum which were suffering from moderate and severe symptoms of toxicity demonstrated normal sex interest when placed with females. This observation is in contrast to that made on young dairy bulls receiving molybdenum (76). The

Figure 1. Young rabbits receiving dietary molybdenum and exhibiting typical alopecia.

fertility of rabbits in this experiment was not determined.

In each of the rabbits which developed the syndrome of toxicity, with two exceptions, the condition became progressively worse until the animal died or was given copper therapy. Two of the young rabbits developed moderately severe symptoms of toxicity but gradually recovered spontaneously. The explanation for this is difficult, but several facts should be observed. The symptoms did not develop in the early weeks of the experiment as was common with the other weanling rabbits, so the animals were older when the abnormalities were observed. The level of molybdenum (0.1 per cent) was borderline for toxicity in mature rabbits. The development of the toxicity was slow and the recovery was slower than the recovery observed with copper treatment.

One of the more specific abnormalities not observed in other species was a "break-down" in the front legs of some young rabbits receiving high levels of molybdenum. This condition occurred in five of the ten weanling rabbits which developed other toxic symptoms. It did not appear in any of the mature rabbits nor any of the other young rabbits after they had passed ten to eleven weeks of age.

In Figure ? are pictures of typical rabbits which exhibit this foreleg abnormality. The first evidence of this condition was an apparent soreness and slight difficulty in walking. There was, however, no evidence of soreness when the legs were examined. The affected animals were observed to sit far back on their rear legs and frequently rest the front legs in the feed cup in apparent effort to shift the body weight from the front feet. They soon became unable

Figure 2. Young rabbits exhibiting front leg abnormality.

to maintain their weight; the legs spread outward and the forepart of the body rested on the floor. The ability to move the legs was not lost and in efforts to walk the legs simply slid on the floor. Two of the rabbits thus affected were treated with copper sulfate when the symptoms of toxicity became severe. There was only slight improvement in this condition while there was complete recovery from all the other symptoms.

Examination of the bone as it was removed from a typical rabbit after sacrificing revealed bending of the upper portion of the humerus and a swollen joint at the junction of the humerus and scapula (Figure 3). Apparently the twisting of the humerus permitted the legs to spread outward. Not all of the cases showed gross bending of the bone or swollen joints and apparently a slight twisting of the bones permitted the legs to spread outward.

Near the termination of this experiment somewhat different abnormalities were observed in the front legs of two young rabbits which died with coccidiosis. These two animals were receiving dietary molybdenum but they have not been included in the results described for the other experimental animals because of the presence of coccidiosis. The infection was accompanied by anorexia, diarrhea and other symptoms which may have complicated the effects of molybdenum.

In the case of one of these rabbits, the feet of both forelegs bent inward at the carpus joint (Figure 2). An examination of the bones as they were removed revealed that the carpus joint of both legs had shifted out of place and the articular surface of the radius was

exposed (Figure 4). The tendons of both joints had slipped out of the normal position. The scapulo-humoral joint of the right leg was loose but had not shifted out of place. There was no gross bending of the bones in this rabbit. In the other young rabbit receiving molybdenum which died with coccidiosis, significant bending of the lower portion of the radius and ulna of one leg was observed. There was also slight enlargement of the scapulo-humoral joint, but no slipped tendons.

Some of the abnormalities of the forelegs of rabbits in this experiment appear to be similar to the crooked front legs described by Smith et al (35, 71) and attributed to manganese deficiency. In the manganese deficient rabbit, the crookedness was confined to the radius and ulna. Gross bending or twisting of these bones has not been a characteristic of the rabbits in this study, although these bones in the leg of one young rabbit were thus affected. The results of this experiment do not, however, eliminate the possibility that a deficiency of manganese is involved and further studies should be made to compare these conditions. Deformities in the legs of rabbits fed a milk diet were observed as early as 1926 by Brouwer (18), but the cause of the abnormality in these rabbits was not known.

In Experiment IV the results of copper therapy are presented. It should be observed at this point, however, that the only symptom of molybdenum toxicity in these rabbits which was not cured by copper therapy was abnormality of the forelegs. Two of the three rabbits which were thus treated exhibited the typical front leg "break-down". The condition in both rabbits improved slightly although all other

Figure 3. Bones from young rabbit showing front leg abnormality.

Figure 4. Forelegs of young rabbit showing shifted carpus joint.

manifestations were completely corrected. The strength of the legs appeared to improve and in the case of one rabbit in which the left leg was not severely affected, it returned to its normal position. The lower photograph of rabbit number 385 in Figure 2 shows the partial recovery following copper therapy. The bone injury appears to be permanent. In attempting to relate this condition to that due to a manganese deficiency, it would be desirable to know whether or not the crookedness in manganese deficient rabbits can be corrected with manganese therapy or whether the injury is permanent.

Two of the characteristic symptoms of molybdenum toxicity in cattle, namely diarrhea and a change in color of the hair coat, have been consistently absent in the syndrome observed in rabbits. In view of the marked alopecia occurring in some animals it is possible that achromotricia may have been masked. It should be observed, however, that achromotricia was not present in those rabbits which were receiving high levels of molybdenum but which did not develop alopecia.

EXPERIMENT II

THE EFFECTS OF VARYING LEVELS OF MOLYBDENUM UPON THE GROWTH OF RABBITS

Common among the symptoms of molybdenum toxicity in species which are subject to the toxic effects is retarded growth or a decrease in body weight. Retarded growth has been observed in rats even though other gross abnormalities were not present. The study of the action of molybdenum upon the growth of young animals is desirable, therefore, in evaluating the effects of sub-lethal quantities of this toxic element. Much of the growth data reported relative to the effects of molybdenum have not been accompanied by adequate feed consumption data so it is difficult to determine whether or not molybdenum per se is the cause of sub-normal growth. The recent experiments of Jeter (49), however, indicate that the retarded growth of rats cannot be attributed to a decreased food intake.

This experiment was designed to determine the relative rates of growth of weanling rabbits fed varying levels of molybdenum.

Experimental Procedure

Twenty-four weanling Dutch rabbits from eight litters were randomized into five treatments consisting of a basal ration and four levels of molybdenum added to the basal ration. The rabbits were six to seven weeks of age and weighed 585 to 820 grams.

The basal ration was the same commercial pellet ration used in Experiment I. The levels of molybdenum added were 0.014, 0.05, 0.1 and 0.2 per cent of the ration, added as sodium molybdate. Rabbits

were housed and managed under the same conditions as outlined in Experiment I.

Growth was determined from weekly weights made throughout the fourteen week growth period. Feed intake measurements were made periodically during the experimental period.

Results and Discussion

The mean weekly weights of the rabbits on the different levels of molybdenum are shown in Table 2. Molybdenum at the level of 0.1 per cent and higher retarded growth. Below this level there was no effect upon the rate of gain. A test of significance for the difference in the amount of gain at twelve weeks for the control group and the group receiving 0.1 per cent molybdenum showed no statistical significance ($P > .05$). At the higher levels of molybdenum, growth was greatly restricted and most of the animals died before a reasonable growth period was completed.

Limited observations show that the rabbits on the higher levels of molybdenum consumed less feed. The intake was not materially reduced, however, until symptoms of the toxicity appeared. There is no evidence that the molybdenum rations were unacceptable, for during the early weeks of the experiment, intakes were essentially normal. After some weeks on the experiment, rabbits receiving the toxic levels of molybdenum were observed to pick over the pellets and to scratch wildly in the feed cups.

When the effects of molybdenum upon the growth of the rabbits are compared with the action of molybdenum in causing anemia, alopecia

TABLE 2

MEAN WEEKLY WEIGHTS OF CONTROL RABBITS AND RABBITS RECEIVING DIETARY MOLYBDENUM
(grams)

Ration	No. Rabbits	Weeks on Experiment: Initial	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	5	738	881	983	1088	1173	1249	1337	1450	1527	1629	1712	1746	1844	1861	1949
0.01%Mo.	5	671	786	888	999	1131	1242	1350	1467	1553	1645	1695	1825	1917	1936	2037
0.05%Mo.	4	700	848	934	1058	1175	1268	1334	1437	1575	1682	1798	1836	1948	2013	2036
0.1%Mo.	5	716	845	978	1083	1145	1187	1218	1371	1424	1524	1611	1529	1521		
0.2%Mo.	5	742	821	868	930	931	952									
0.02%Cu. 0.2%Mo.	2	710	816	941	1084	1145	1235	1316	1450	1514	1552	1697	1619	1766	1799	1886

and other toxic symptoms, it can be observed that growth was retarded only in those animals which developed the toxicity syndrome. When the toxic symptoms were absent, even though the rabbits were receiving molybdenum, growth was not retarded. This observation is in contrast with the effect of molybdenum upon rats where growth has been retarded in the absence of other gross abnormalities.

In evaluating the effects of molybdenum in this experiment, it is necessary to consider the relatively high level of copper (16.4 ppm) in the basal ration. With this intake of copper a greater tolerance for molybdenum would be expected. The action of molybdenum in retarding growth is, however, demonstrated. Since the consumption of feed was somewhat less in the groups in which growth was sub-normal, it cannot be determined whether or not molybdenum per se retards growth or reduces the feed intake.

EXPERIMENT III

THE EFFECTS OF VARYING LEVELS OF MOLYBDENUM UPON THE HEMOGLOBIN, RED BLOOD CELL COUNT AND BLOOD VOLUME OF RABBITS

Anemia is common in the syndrome of molybdenum toxicity in cattle and sheep. The investigations which have related anemia to excess molybdenum have not included intensive studies relative to other changes in the blood picture. Included in this experiment are observations of the changes in hemoglobin, red blood cell count and blood volume of normal rabbits and of rabbits suffering from molybdenum toxicity.

Experimental Procedure

Blood analyses were made on the rabbits in Experiments I and II. The analyses were made concurrently with the development of the toxic syndrome and with the observations upon the growth of the rabbits. No alterations were made in the feeding or management procedure.

Blood samples were taken from the marginal ear vein and hemoglobin determined every two weeks on the experimental animals using the acid hematin method (20). When the first symptoms of toxicity appeared, determinations were made more often in order to follow more closely the changes in hemoglobin.

Red blood cell counts were made on the blood from representative control rabbits and those receiving high levels of molybdenum before and after the hemoglobin began to decrease. Cells were counted

in the standard red cell counting chamber after dilution in Hayem's solution.

The blood volume of two control rabbits and of six rabbits receiving varying levels of dietary molybdenum was determined. The determinations were made with phosphorus^{32} labelled cells (45) and also by the dye dilution method (44, 62). The rabbits were given ether anaesthesia and both jugular veins were exposed. Injections and withdrawals were made from opposite veins and the calculations based upon samples of blood taken after five and ten minute intervals.

Results and Discussion

The mean hemoglobin values which were obtained from rabbits on the control ration and the different levels of molybdenum are presented in Table 3. These averages do not represent values from all rabbits in each group, but are calculated from determinations made on three to five rabbits in each treatment which were representative of the group. The values obtained for the control rabbits and those receiving the lower intakes of molybdenum (0.014 and 0.05 per cent) were within the normal range for this species (23). The rabbits receiving 0.1 per cent or more molybdenum in the ration developed anemia as evidenced by the low hemoglobin. Since the values reported in Table 3 are averages, the extremely low hemoglobin which was found in some animals is not evident. In Table 4 are shown the hemoglobin values for some individual rabbits as anemia developed. Anemia was first observed at about the same time that other symptoms of toxicity became evident and the hemoglobin continued to decrease

until death occurred or until copper therapy was initiated. Hemoglobin as low as 2.0 grams per 100 ml blood has been observed before death occurred.

The number of erythrocytes in the blood of normal rabbits and experimental rabbits before the toxic syndrome developed were within the normal range for rabbits (23). When anemia appeared and progressed, there was a corresponding decrease in the red blood cell count. In Table 5 are hemoglobin values and red blood cell counts of some individual rabbits. These data relate the hemoglobin measurements to the number of erythrocytes and further relate the anemia to dietary molybdenum.

TABLE 3

MEAN HEMOGLOBIN VALUES FOR EXPERIMENTAL RABBITS*

Week on Experiment	Per cent molybdenum in ration				
	Basal	0.014	0.05	0.1	0.2
Initial	12.5	12.1	12.1	11.0	12.6
2	12.9	12.6	12.3	12.5	13.2
4	13.0	11.3	12.8	[illegible].5	[illegible].6
6	12.8	11.3	[illegible].9	[illegible].1	7.6
8	13.8	**	12.5	[illegible].0	11.8
10	12.1	[illegible].1	12.1	7.6	13.3
12	12.9	13.3	12.6	8.7	10.9
14	13.[illegible]	13.8	13.0	10.8	7.3
16	14.[illegible]		14.8	[illegible].5	5.7
18	12.4	16.2	16.2	10.6	

* Grams hemoglobin per 100 ml blood.
** Values not determined.

TABLE 4

HEMOGLOBIN OF SOME INDIVIDUAL RABBITS RECEIVING DIETARY MOLYBDENUM

Rabbit No.	Mo. in ration	Grams hemoglobin per 100 ml blood						
333	0.1%	(7)*14.0	(8) 12.0	(10) 8.0	(12) 3.5**			
343	0.1%	(7) 12.8	(9) 7.5	(10) 5.0	(12) 7.3	(13) 8.8	(14) 9.8	(15) 7.8
353	0.1%	(3) 11.0	(5) 10.5	(7) 6.3	(8) 6.0	(10)10.0	(11)10.3	(12)13.3
382	0.1%	(3) 13.0	(5) 6.2	(5+1 day) 5.4**				
385	0.1%	(1) 12.0	(3) 9.0	(3+1 day) 8.0	(3+2 days) 7.5**			
335	0.2%	(2) 12.5	(8) 10.7	(4) 5.5	(5) 2.7	dead		
344	0.2%	(9) 14.2	(12) 9.2	(13)10.3	(14)10.3	(15) 8.2	(16) 6.1	dead
386	0.2%	(11)12.9	(12)12.6	(13) 7.6	(14) 4.3	(14+1 day) 3.2	dead	
909	0.4%	(5) 14.0	(6) 11.3	(7) 8.8	(8) 7.0	(9) 4.5	(9+1 day) 4.0	dead

* Numbers in parenthesis represent weeks on experiment.
** Copper therapy initiated.

TABLE 5

HEMOGLOBIN AND RED BLOOD CELL COUNTS OF INDIVIDUAL RABBITS

Rabbit No.	Ration	Week of Expt.	Hemoglobin*	Erythrocytes** (millions)
331	Control	12	13.2	6.16
341	Control	17	15.0	6.42
301	Control	17	14.5	5.79
906	Control	17	12.0	5.08
304	0.014% Mo.	8	16.0	6.11
372	0.014% Mo.	23	13.1	6.29
332	0.05% Mo.	6	15.0	6.20
342	0.05% Mo.	17	13.0	6.40
333	0.1% Mo.	13	3.5	1.55
343	0.1% Mo.	17	10.7	5.54
353	0.1% Mo.	7	6.3	3.39
382	0.1% Mo.	5	6.3	3.16
385	0.1% Mo.	3	8.0	3.40
335	0.2% Mo.	4	5.5	1.89
344	0.2% Mo.	17	7.3	3.50
386	0.2% Mo.	13	3.2	1.20
909	0.2% Mo.	9	4.5	1.53

* Grams per 100 ml Blood.
** Cells per cubic millimeter.

Limited measurements of the red blood cell size indicated that erythrocytes were smaller and more variable in size in anemic rabbits than in normal rabbits.

The blood volume for the control rabbits and experimental rabbits is recorded in Table 6. Other data relative to the blood picture which were obtained at the same time are also presented. The relationship of the plasma volume to the red cell count is also reflected in the low hemoglobin and red cell count. The blood volume of all of the rabbits is within the normal range for the age and weight of the animal. It is significant that blood volume was maintained at a normal level even though severe anemia was present. The results indicate, therefore, that the mechanism for maintaining blood volume is not affected in molybdenum toxicity.

TABLE 6

BLOOD VOLUME, HEMOGLOBIN AND RED BLOOD CELLS OF NORMAL RABBITS AND RABBITS ANEMIC WITH MOLYBDENUM TOXICITY

Rabbit No.	Ration	Hemoglobin	Erythrocytes (million)	Dye Method			P^{32} Method		
				P.V.	R.C.V.	ml blood/ 100 gm wt	P.V.	R.C.V.	ml blood/ 100 gm wt
906	Control	12.0	5.08	78.0	50.6	4.63	63.4	41.4	5.66
200	Control	14.0	6.46			*	73.5	42.6	5.10
372	0.014%Mo	13.1	6.29	68.3	33.8	4.77			**
342	0.05%Mo	12.0	6.43	86.8	48.8	6.08	98.9	55.7	6.93
343	0.1%Mo	10.7	5.54	91.8	53.7	7.47	96.0	54.8	7.74
392	0.1%Mo	2.0	0.9	48.8	2.8	5.65			
344	0.2%Mo	7.3	3.5	79.4	21.6	5.54	87.4	24.5	6.13
386	0.2%Mo	3.2	1.2	70.6	8.2	6.24			

* Dye method not employed.
** P^{32} method not employed.

EXPERIMENT IV

THE ROLE OF COPPER IN RELATION TO MOLYBDENUM TOXICITY

In considering the role of molybdenum in animal nutrition, it is now evident that its effects must be related to the copper intake and copper status of the animal. The therapeutic action of copper upon the copper status of the animal has already been reviewed. Included in this experiment are observations on the prevention of molybdenum toxicity with dietary copper, the therapeutic action of copper, and the relation of molybdenum to liver storage of copper in the rabbit.

Experimental Procedure

Two weanling Dutch rabbits, six to seven weeks of age and weighing 628 and 791 grams, were placed on a ration containing .02% copper and 0.2% molybdenum. The basal ration was the commercial rabbit pellets used in prior experiments. Feeding and management procedures were the same as previously outlined. Daily observations were made for appearance of any abnormalities and body weights made weekly for growth estimation. Copper as $CuSO_4 \cdot 5H_2O$ and molybdenum as $NaMoO_4 \cdot 2H_2O$ were sprayed onto the ration from aqueous solutions. It was necessary to add each of the salts to separate portions of the ration then mix after drying.

In order to further observe the therapeutic effect of copper, three rabbits which were receiving high levels of molybdenum and had developed the typical toxicity syndrome were treated with copper. The

clinical manifestations were allowed to progress until it appeared that the animals would not longer survive without treatment. At this time they were given a drench of 0.05 gm $CuSO_4 \cdot 5H_2O$ in 10 ml water and thereafter they were supplied drinking water which contained 0.1 gm $CuSO_4 \cdot 5H_2O$ per 500 ml water. Clinical observations were continued in order to follow the changes resulting from copper treatment.

At the termination of the experiment, rabbits were killed or sacrificed for phosphorus balance and distribution studies. Livers and femur bones were removed and copper storage in the liver determined using the carbamate method (66).

Results and Discussion

Neither of the rabbits which was given supplementary copper and molybdenum in the ration developed any of the symptoms of molybdenum toxicity. The rate of gain was slightly less but not significantly lower than the control rabbits (Table 2). The level of molybdenum in this ration (0.2%) had proved to be toxic in the absence of added copper so the protection afforded by the copper is demonstrated.

From other observations made in this phase of the experiment, an estimation of the ratio of copper required for protection may be made. When the copper sulfate solution was added to the ration, this portion turned distinctly blue-green in color so that even when mixed with the normal colored molybdenum treated portion, the copper pellets were easily distinguishable. The rabbits picked over the ration, leaving a large portion of the copper treated pellets. Because of the

difference in color, it could be observed that much less than the calculated amount of added copper was actually consumed. Therefore, the amount of copper required to protect against the 0.? per cent molybdenum was much less than 0.02 per cent added copper and more than the 0.0016 per cent contained in the basal ration.

The three rabbits which had severe symptoms of toxicity and were treated with copper responded rapidly to the copper therapy. In Table 7 data are presented illustrating some of the changes which were observed following the initiation of copper treatment.

Not shown in the table are the effects of the treatment upon alopecia and dermatosis. Dermatosis disappeared and the hair began to return to the denuded areas within about a week.

The return to normal weight gains, normal hemoglobin and full hair coat was generally complete within one month. There was only slight improvement in the foreleg abnormality after five months treatment with copper. The failure of this condition to respond to copper has been discussed in Experiment I.

The relative accumulation of copper in the livers of rabbits on the different dietary levels of molybdenum is shown in Table 8. The higher concentrations of copper in the livers from those rabbits which received supplementary molybdenum show that molybdenum had a tendency to cause an increase rather than a decrease in the copper storage. The concentration of copper in the livers from those rabbits receiving supplemental molybdenum and copper was considerably higher than that found in any of the other groups. This intake of copper

TABLE 7

EFFECTS OF COPPER THERAPY UPON WEIGHT, HEMOGLOBIN LEVEL, AND RED CELL COUNT OF RABBITS AFFECTED WITH MOLYBDENUM TOXICITY

Rabbit No.	Time after treatment	Weight	Hemoglobin	Erythrocytes (millions)
333	Initial	1144	3.5	
	1 week	1137	7.0	
	2 weeks	1204	10.0	
	3 weeks	1362	10.8	
	5 weeks	1448	12.6	
382	Initial	853	5.4	
	1 week	915	9.0	
	3 weeks	1080	12.7	
	5 weeks	1276	13.0	
385	Initial	907	7.5	3.75
	1 day	—	8.0	4.15
	3 days	871	7.5	3.80
	1 week	944	10.5	4.69
	2 weeks	993	11.0	
	4 weeks	1296	10.3	

was not considered a high level but was estimated to be the amount necessary to prevent the toxic action of molybdenum. The quantity of copper found in the livers of the control rabbits is slightly greater than the amount reported by Lorenzen and Smith (54) and is considerably higher than the quantity reported by Cunningham (24).

Although the liver copper was not greatly increased except in the group receiving supplemental copper, it is significant that molybdenum did not cause a decrease in the copper content of the liver. This finding supports previous observations (51, 58) that molybdenum tends to cause an accumulation of copper when the intake of copper is adequate.

TABLE 8

ACCUMULATION OF COPPER IN LIVERS OF EXPERIMENTAL RABBITS ON DIFFERENT MOLYBDENUM AND COPPER INTAKES

Ration	No. of Rabbits	Micrograms Cu per gram dry liver (average)
Control	5	29.7
0.014% Mo.	5	30.2
0.05% Mo.	3	45.0
0.1% Mo.	5	35.7
0.2% Mo. 0.02% Cu.	4	60.5

EXPERIMENT V

THE EFFECTS OF LOW INTAKES OF DIETARY MOLYBDENUM AND OF VITAMINS A AND E IN THESE DIETS UPON THE GROWTH AND REPRODUCTION OF RATS

The results from several investigations of the role of molybdenum in the rat show that the element is toxic, causing death at high levels and retarded growth at sub-lethal levels. Previous work in this laboratory has shown that 80 and 140 ppm molybdenum in a simplified ration significantly retards growth. Observed also in this experiment was an interference with reproduction (49). From these two observations it appeared that there might be a deficiency of vitamin A or vitamin E as a result of the action of molybdenum. If the vitamins become deficient by being tied up in the alimentary tract, or are otherwise rendered ineffective by molybdenum, the deficiency should be corrected by therapeutic administration of the vitamins. This experiment was designed to further study the effects of low intakes of molybdenum upon growth and reproduction of rats and to study the effects of vitamins A and E added to diets containing molybdenum.

Experimental Procedure

Ninety weanling rats of the Sherman strain, 21 to 23 days old, were randomized into three treatments of 30 rats each. These treatments consisted of the basal ration and two levels of molybdenum (0.014 and 0.02 per cent) added to the basal ration. These three treatments were further subdivided into three sub-groups of 10 rats

each approximately equally distributed between males and females. One of the sub-groups in each treatment was considered the basal treatment for the addition of the vitamin A or vitamin E to each of the other two sub-groups.

The rats were housed in metal wire cages maintained in an air-conditioned room at 80° F. Rations were fed ad libitum and pyrex-distilled water was provided from glass watering bottles.

The composition of the basal ration is recorded in Table 9. The whole milk powder was Powdered Klim, received in vacuum packed containers. Rations were prepared fresh about each ten days. The copper content of this ration was 5 ppm. Vitamins A and E were administered directly to the rats with the use of a medicine dropper. The vitamins were given twice weekly in amounts that supplied 5,000 units of vitamin A and 12 milligrams of alpha tocopherol in each dose. Vitamin A was in the form of a natural ester concentrate and vitamin E was alpha tocopherol acetate.

Growth was determined from weekly weights made throughout the twelve-week growth period. At 16 weeks of age females were bred to males on the same ration and any rats which did not breed were later exposed to other rats to check the cause of the breeding failure.

In order to observe the protective action of copper against the effects of molybdenum, one ration was prepared which contained 0.02% molybdenum and 0.002% copper. The basal ration for the trial was the same as previously used. The composition of both is shown in Table 9. Five male and four female weanling rats were used in this

trial and procedures previously outlined were used for obtaining data. These rats were not given vitamins.

TABLE 9

COMPOSITION OF RATIONS
(grams)

	Basal	Basal + 0.014% Mo.	Basal + 0.02% Mo	Basal + 0.002% Cu 0.02% Mo
Whole Milk Powder (Klim)	3415.0	3415.0	3415.0	3415.0
Sucrose	3380.0	3380.0	3380.0	3380.0
NaCl	34.0	34.0	34.0	34.0
$FeSO_4 \cdot 7H_2O$	4.0	4.0	4.0	4.0
$MnSO_4 \cdot H_2O$	2.52	2.52	2.52	2.52
Thiamine hydrochloride	0.023	0.023	0.023	0.023
Pyridoxine hydrochloride	0.033	0.033	0.033	0.033
$CuSO_4 \cdot 5H_2O$	0.10	0.10	0.10	0.528
$NaMoO_4 \cdot 2H_2O$	0.0	2.38	3.43	3.43

Results and Discussion

The mean weekly weights of the rats throughout the 12 week growth period are recorded in Tables 10 and 11. Weights of the male rats are found in Table 10 and females in Table 11. Also included in the table are the average total gains for each group. The growth of the male rats receiving molybdenum was significantly less than the growth of those in the basal group. The difference in the amount of gain for the group receiving 0.014 per cent molybdenum and of the basal group was highly significant ($P<.01$). For those receiving 0.02 per cent molybdenum, the gain was significantly less ($P<.05$).

TABLE 10

MEAN WEEKLY WEIGHTS AND TOTAL GAIN OF MALE RATS
(grams)

Ration	Weeks													Total Gain
	Initial	1	2	3	4	5	6	7	8	9	10	11	12	
Basal	42	60		96	107	128	150	167	185	202	218	229	237	195
Basal + Vitamin A	45	64	80	99	117	150	171	192	212	230	245	263	273	228
Basal + Vitamin E	43	60	65	95	116	141	161	178	200	217	238	241	253	210
0.014% Mo	41	61	72	95	110	128	134	166	182	199	210	226	235	194
0.014% Mo + Vitamin A	36	46	66	83	96	110	123	138	148	158	177	193	202	166
0.014% Mo + Vitamin E	35	45	56	73	85	96	112	123	138	154	163	176	179	144
0.02% Mo	45	62	83	97	117	137	147	168	184	204	221	224	233	188
0.02% Mo + Vitamin A	43	56	74	88	105	123	141	160	178	191	205	217	226	183
0.02% Mo + Vitamin E	43	58	73	95	113	131	133	163	169	191	194	215	224	181
0.002% Cu 0.02% Mo	40	51	69	85	97	115	131	152	177	186	190	209	224	184

TABLE 11

MEAN WEEKLY WEIGHTS AND TOTAL GAIN OF FEMALE RATS
(grams)

Ration	Weeks													Total Gain
	Initial	1	2	3	4	5	6	7	8	9	10	11	12	
Basal	40	51	54	80	90	108	120	128	140	147	153	161	168	128
Basal + Vitamin A	44	64	86	104	123	147	169	175	185	190	197	205	211	168
Basal + Vitamin E	45	59	78	96	120	138	151	163	172	178	183	195	201	156
0.014% Mo	40	57	68	89	97	109	121	134	144	153	180	167	173	133
0.014% Mo + Vitamin A	38	57	72	98	105	117	122	148	157	166	176	183	190	152
0.014% Mo + Vitamin E	39	55	73	89	104	124	120	152	161	171	178	185	192	153
0.02% Mo	40	54	73	88	98	110	127	135	144	151	158	167	171	131
0.02% Mo + Vitamin A	42	59	78	99	120	138	153	169	179	187	192	200	204	162
0.02% Mo + Vitamin E	41	57	74	89	106	119	129	147	155	159	167	175	180	139
0.002% Cu 0.02% Mo	41	55	73	83	106	120	137	148	159	167	173	177	183	142

The growth of the female rats receiving molybdenum appeared to be retarded, but the differences in amount of gain were not statistically significant for either level of molybdenum. This sex difference in response to molybdenum supports a previous observation (49) that male rats are less tolerant to dietary molybdenum than are female rats.

The effects of the vitamins may also be observed in Tables 10 and 11. The addition of both vitamins A and E increased the growth of female rats on all treatments including the basal ration. No consistent differences in the growth of male rats could be attributed to either vitamin. The objective of the vitamin supplementation was to study its possible therapeutic effect upon molybdenum toxicity. Although both vitamins appeared to increase the growth of the female rats on both levels of molybdenum, they had a like effect upon the females on the basal ration; therefore, the relation to molybdenum could not be observed. In the male rats where the adverse effects of molybdenum were more evident, it should be observed that neither vitamin A nor vitamin E increased the growth. From this observation it appears, therefore, that neither vitamin A or vitamin E is effective in counteracting molybdenum toxicity.

The average weekly weights and total gain of the rats given 0.002 per cent copper and 0.02 per cent molybdenum are recorded in Tables 10 and 11. This amount of copper was not considered a high level of dietary copper but was the quantity estimated to be necessary to counteract the effects of molybdenum. This ration, however, did not support growth in the male rats equal to that of the controls. The

amount of gain was significantly less than the gains produced in control male rats. There was no significant difference in the growth of these female rats. The growth of these females which was approximately equal to that of the control females cannot be attributed to the presence of the additional copper since the female rats which received molybdenum but without the additional copper made approximately equal gains.

The results of the breeding trials are summarized in Table 12.

TABLE 12

FERTILITY OF MALE AND FEMALE RATS RECEIVING DIETARY MOLYBDENUM AND COPPER

Ration	Males			Females		
	No. Matings	No. Litters	No. Sterile Males	No. Matings	No. Litters	No. Sterile Females
Basal	6	6	0	5	5	0
0.014% Mo	6	6	0	4	4	0
0.02% Mo	5	4	1	5	5	0
.002% Cu .02% Mo	5	5	0	4	4	0

From these results, no significant effect of the amount of molybdenum fed can be observed. The proportion of fertile rats in the groups receiving supplemental vitamins A and E was the same. Since the molybdenum did not produce sterility in this experiment, the effects of the vitamins upon reproduction cannot be determined. These results are contrary to the results of fertility experiments with rats (49).

wherein a large proportion of male rats on a similar dietary regime were sterile. A higher level of molybdenum has been fed in the current experiment without causing sterility. The basal rations and other conditions of these two experiments were similar; however, the following changes applied to the current experiment may account for the differences in results: (1) Sherman (albino) rats were used in this experiment. The Long-Evans strain was used in the former experiment. (2) The iron and manganese content of the ration was increased. (3) Pyridoxine was added to the ration. These changes apparently improved the ration, as reflected in somewhat better growth of the animals, and with an improved nutritional status of the animals, the greater tolerance to molybdenum would be expected.

During the course of the experiment, a peculiar alopecia was observed in some of the male rats receiving molybdenum and also in those receiving the higher level of copper with molybdenum. It was not present in all of the rats of any group nor was the loss of hair permanent. This alopecia was much more common in the male rats and was limited to a strip approximately an inch wide over the entire length of the back. Figure 5 shows pictures of typical rats exhibiting this alopecia. Alopecia appeared in some of the other rats, particularly about the neck and shoulders, but it was not confined to the rats receiving molybdenum, nor was it observed more in male rats than in females.

The alopecia which occurred over the back of the animals appeared to be associated with molybdenum. Although less than one-third

of the male rats on the molybdenum diets exhibited this particular type of alopecia, none of the rats of the basal group were so affected. Alopecia has been observed in rats on similar diets, but it was more characteristic of the female rats than of males (49).

Figure 5. Male rats on molybdenum diets exhibiting alopecia over the back.

EXPERIMENT VI

PHOSPHORUS METABOLISM AND DISTRIBUTION OF PHOSPHORUS^{32} IN THE RABBIT AND RAT

Certain abnormalities in skeletal formation have been attributed to excess molybdenum in the dietary. Some of the investigations which have been undertaken to relate this abnormality to molybdenum have demonstrated an abnormal phosphorus metabolism when molybdenum was in excess. The observation made early in this experiment that the front legs of some young rabbits develop abnormally when fed high levels of molybdenum demonstrated that molybdenum interferes in some manner with normal skeletal development in this species. In the absence of other explanation, it appeared possible that there was an interference with phosphorus metabolism.

This experiment was undertaken to obtain data which would afford an evaluation of phosphorus balance in normal rabbits and rats and in rabbits and rats which were receiving dietary molybdenum. Also included in the study are observations on the distribution of radioactive phosphorus in these animals.

Experimental Procedure

The animals used in this study were selected from those used in previous experiments. They were continued on the same ration after completion of the growth studies. Four young rabbits and 18 young rats were introduced into the experiment at this time in order to obtain data from young animals.

Phosphorus balance studies were made using radioactive phosphorus^{32}.

Following administration of the isotope, rabbits were confined for 72 hours to metabolism cages which provided quantitative separation of urine and feces. The 72 hour period was selected because preliminary observations indicated that the greater portion of the isotope was excreted within this time. Ten rabbits were given oral administration of the isotope and seven were given intravenous injections through the marginal ear vein. Administration to the rats was made orally and collection periods were 48 and 72 hours.

At the end of the metabolism period, the animals were sacrificed and selected tissues taken for distribution studies. Urine and feces were collected and the radioactivity determined on representative samples.

The radioactive phosphorus which was received in the form of phosphoric acid was neutralized with sodium carbonate and made isotonic with sodium chloride prior to administration. The dosage administered to rabbits ranged from 1.5 to 5 microcuries and the rats were given 1 to 2 microcuries.

Radioactivity measurements were made in the usual manner using a Geiger-Müller counter fitted with a dipping tube. Urine and blood samples were counted directly without ashing. Before determining the activity of the rabbit urine, it was necessary to acidify the urine with hydrochloric acid in order to dissolve the solid portion of the urine. This portion was observed to contain a large proportion of the phosphorus and unless it was dissolved or thoroughly suspended, the measurements were inaccurate. Feces and tissue samples were wet

digested with nitric acid and heat prior to activity measurements.

Calculations were made of the per cent of total dose excreted in the urine and feces during the balance period. The per cent of total dose per gram of tissue was determined for the liver, kidney, femur, muscle and blood and the per cent of dose was corrected to the average body weight of the animals.

The total phosphorus content of femur bones and certain other tissues was determined using a modification of the volumetric method outlined in Methods of Analysis of the Association of Official Agricultural Chemists (6).

Results and Discussion

Balance Studies — The data representing the balance studies with rabbits are summarized in Table 13. The values reported are mean values for the per cent of total dose which was excreted in 72 hours by way of the urine and the feces. Results are given for both oral and intravenous administration of the isotope. The results show that a greater proportion of the orally administered phosphorus^{32} was excreted by the rabbits which were receiving dietary molybdenum. There was a significant increase in both urinary and fecal excretion of the labeled phosphorus. Following intravenous administration, there was no observed difference in the total amount excreted nor in the pathway of excretion. This indicates that in the rabbit molybdenum did not alter the pathway of excretion as has been observed in steers (68). On the high molybdenum rations, the urinary excretion of total phosphorus increased almost fourfold while fecal excretion of total

TABLE 13

EFFECTS OF DIETARY MOLYBDENUM AND METHODS OF ADMINISTRATION UPON THE EXCRETION OF PHOSPHORUS^{32} AND OF TOTAL PHOSPHORUS BY THE RABBIT

Ration	% of total dose of Phosphorus^{32} excreted						Mg total P excreted average 3 rabbits		
	Oral Administration			Intravenous Administration					
	Urine	Feces	Urine + Feces	Urine	Feces	Urine + Feces	Urine	Feces	Urine + Feces
Control	4.80	39.44	44.24	11.27	8.45	19.72	49.62	744.0	793.63
High Mo.	8.95	52.69	61.64	10.64	8.95	19.59	195.60	614.0	809.60

phosphorus was not significantly affected. The increased excretion of both labeled and total phosphorus by way of the urine in animals on high molybdenum diets indicates that molybdenum may decrease the absorption of phosphorus and at the same time increase the excretion of that which has been absorbed. Since there was no significant alteration in the excretion of labeled phosphorus following intravenous administration, it appears that the primary effect of molybdenum is in the gastro-intestinal tract.

The excretion data for the rats on control and high molybdenum diets are presented in Table 14. These values represent only oral administration of the isotope. Results from both mature and young rats and of two intervals of time after administration are recorded. The amount of total phosphorus in the urine could not be accurately determined because of contamination from feed. The total excretion of phosphorus^{32} was not increased by molybdenum feeding in the case of the rats. There was an increase in urinary excretion of the labeled phosphorus, but this was not accompanied by an increased excretion through the feces. The effect of molybdenum upon phosphorus^{32} excretion in the urine parallels the results observed with oral administration to rabbits. Both total phosphorus and labeled phosphorus were less in the feces from the rats receiving molybdenum. The urinary excretions at 48 and 72 hours indicated that molybdenum in the young rat caused more rapid excretion of the absorbed labeled phosphorus. There was no increase at 72 hours over that excreted at 48 hours. With mature rats, the excretion at 72 hours was almost double that at

48 hours. This is also reflected in the fecal excretion but to a lesser degree.

TABLE 14

EFFECTS OF DIETARY MOLYBDENUM, AGE AND TIME AFTER DOSING UPON THE EXCRETION OF ORALLY ADMINISTERED PHOSPHORUS^{32} BY THE RAT

Ration	Hours after Dose	Age (weeks)	No. Rats	Urine	Feces	
				% Dose	% Dose	Mg total P
Control	48	25	4	8.60	27.97	47.95
		8	4	6.98	16.06	50.45
	72	25	2	15.72	33.59	66.63
		8	2	18.73	14.97	33.87
High Mo.	48	25	4	12.19	15.65	36.55
		8	4	12.07	6.01	38.11
	72	25	2	22.57	25.25	55.78
		8	3	13.49	12.70	38.47

Tissue Distribution — Data representing the deposition of labeled phosphorus in the tissues under the different conditions are summarized in Tables 15, 16 and 17. The values reported have been corrected to the average weight of the animals within the groups for which the comparisons are made.

The deposition of phosphorus^{32} was very similar for comparable tissues of the animals on different rations and there were only slight differences which may be attributed to the effects of molybdenum. The

per cent of dose of the labeled phosphorus was slightly higher in the liver and kidney of both the rats and rabbits which were on the high molybdenum diets. The femur bone of the rabbits receiving molybdenum accumulated a greater percentage of the administered dose than did the control rabbits, but no similar increase was observed in the rats.

TABLE 15

EFFECTS OF MOLYBDENUM UPON THE DEPOSITION OF PHOSPHORUS^{32} IN SELECTED TISSUES OF THE RABBIT*

Tissue	Per Cent of Dose per Gram of Tissue			
	Oral Administration (3 rabbits)		Intravenous Administration (2 rabbits)	
	Control	High Mo.	Control	High Mo.
Blood	0.016	0.012	0.018	0.014
Liver	0.105	0.125	0.148	0.172
Kidney	0.085	0.087	0.109	0.126
Muscle	0.026	0.019	0.044	0.070
Femur shaft	0.149	0.154	0.136	0.271
Femur epiphysis	0.255	0.315	0.272	0.488

* 72 hours after administration.

The effects of molybdenum and of two intervals of time after administration upon accumulation of phosphorus^{32} in the tissues of young rats are shown in Table 17. Also shown is the amount of total phosphorus in the femurs of these rats. The per cent of the total dose

TABLE 16

EFFECTS OF DIETARY MOLYBDENUM UPON DEPOSITION OF PHOSPHORUS^{32} AND TOTAL PHOSPHORUS IN SELECTED TISSUES OF MATURE RATS*

Tissue**	Control			High Mo.		
	% Dose Per gm. Tissue	% Dose Per mg. P.	mg. Total P.	% Dose Per gm. Tissue	% Dose Per mg. P.	mg. Total P.
Blood	0.019		***	0.030		***
Liver	0.595	0.35	2.37	0.638	0.34	2.69
Kidney	0.116	0.066	2.61	0.118	0.064	2.67
Muscle	0.080	0.057	2.34	0.037	0.030	2.29
Femur	1.17	0.020	85.87	1.167	0.019	85.89

* 48 hours after administration.
** Tissues represent pooled samples of 4 rats.
*** Total phosphorus not determined.

TABLE 17

DEPOSITION OF PHOSPHORUS^{32} AND AMOUNT OF TOTAL PHOSPHORUS IN SELECTED TISSUES OF YOUNG RATS*

Tissue	Hours after Dose	Per Cent of Dose per Gram of Tissue	
		Control	High Mo.
Blood	48	0.026	0.029
	72	0.044	0.043
Liver	48	0.456	0.507
	72	0.375	0.363
Kidney	48	0.211	0.226
	72	0.378	0.388
Muscle	48	0.089	0.085
	72	0.203	0.217

		% Dose	% Dose Per mg. P.	mg. total P.	% Dose	% Dose Per mg. P.	mg. total P.
Femur	48	2.81	0.046	60.33	2.96	0.058	56.96
	72	3.49	0.057	60.90	3.86	0.080	47.75

* Tissues represent pooled samples of 4 rats at 48 hours and 3 rats at 72 hours.

of labeled phosphorus found in the tissues at 72 hours was greater than that found at 48 hours in all of the tissues except the liver. These increases were common to the rats on both rations; therefore, no specific effect of molybdenum upon the rate of accumulation can be observed in the young rats. The increase in the labeled phosphorus of the kidney at 72 hours is also reflected in the urinary excretion at 72 hours.

The decrease in the amount of total phosphorus and the increased specific activity of the bone of young rats receiving molybdenum (Table 17) indicate a greater rate of exchange of phosphorus and more interference with normal phosphorus deposition in the young rat than in the mature rat.

When the concentration of labeled phosphorus per unit of total phosphorus was calculated there was little difference in the specific activity of the tissues of rats on the different rations (Table 16). The difference in the per cent dose per milligram of total phosphorus in the bone of old and young rats reflects the larger amount of total phosphorus present in the bone of mature rats.

There appears to be a species difference in the tissue accumulation of labeled phosphorus. The soft tissues and the femur bones of the rats accumulated a considerably greater proportion of the dose of the isotope per unit of tissue than did the rabbit tissues. A portion of this difference may be attributed to the difference in time after administration of the isotope. Experiments involving a large number of animals should be undertaken in order to establish whether such a

difference exists.

The total phosphorus content of the femur bones from 17 rabbits receiving different dietary levels of molybdenum has been determined. The results which represent mean values for the number of bones examined are summarized in Table 18. There were wide variations in individual rabbits, but the averages indicate a slight decrease in the phosphorus content of the bones of rabbits which were receiving molybdenum. Two of the rabbits in the group receiving copper and molybdenum had previously received molybdenum without copper supplementation. They had developed typical symptoms of toxicity, one of which exhibited the foreleg trouble. Both rabbits were treated with copper and the copper supplementation was continued until they were sacrificed and the total phosphorus of the bones determined.

TABLE 18

TOTAL PHOSPHORUS CONTENT OF FEMUR BONES OF RABBITS FED VARYING LEVELS OF MOLYBDENUM AND COPPER
(mg per gram fresh tissue)

Ration	No. of Rabbits	mg P. per gram Tissue
Control	4	96.16
0.01% Mo.	3	94.94
0.1% Mo.	6	87.93
0.2% Mo. + 0.02% Cu.	4	89.96

It appears that the effects of molybdenum upon the metabolism of phosphorus under the conditions of this experiment were limited to alteration in the excretion of phosphorus. While there were slight changes in the deposition of labeled phosphorus in some of the tissues, these differences were not significant and not as pronounced as the changes in excretion which have been described. It should be noted that none of the animals exhibited symptoms of molybdenum toxicity at the time when these phosphorus metabolism studies were undertaken. The quantity of molybdenum (0.1%) fed to the rabbits in this experiment had produced toxicity in other young rabbits and had produced moderate symptoms in some of the rabbits which were later used for these phosphorus studies, but none exhibited any toxic symptoms at the time of this experiment. If the phosphorus studies had been made when evidence of molybdenum toxicity first appeared, it is possible that the effects of molybdenum may have been exhibited in a different manner.

GENERAL SUMMARY

Summary and Conclusions

The role of molybdenum in the nutrition of the rabbit has been studied through observations of the effect of supplementary molybdenum in the diet. A molybdenum toxicity was produced and the symptoms have been described. Anemia was characteristic of the toxic syndrome and was further studied with respect to hemoglobin, red blood cell counts and blood volume. The therapeutic action of copper in curing the toxicity was studied by following the changes resulting from copper therapy. Growth experiments were conducted with weanling rabbits and rats receiving varying levels of molybdenum. Some effects of molybdenum upon the metabolism of phosphorus were studied through balance experiments and tissue distribution of radioactive phosphorus^{32}.

Some of the results have been of a confirmatory nature while other findings have demonstrated actions of molybdenum which appear to be specific for the rabbit. These observations have been related to results of experiments with other species and have been discussed with respect to their possible implications. Conclusions which may be made from these experiments are summarized in the following statements:

1. Molybdenum is toxic to rabbits. Under conditions of this experiment, 0.1 per cent added to a natural ration was toxic to young rabbits and was borderline in toxicity for mature rabbits. Two-tenths and 0.4 per cent molybdenum was toxic to all ages of rabbits.

2. Molybdenum toxicity in the rabbit was characterized by loss of weight, anemia, alopecia and dermatosis and abnormal bone development.

3. The abnormal bone development was observed in young rabbits receiving 0.1 per cent or more molybdenum. The front legs of affected rabbits became weakened so that they could not be maintained in a normal position and could not support the body. Observed in the abnormality were swollen joints, bent and twisted bones, slipped joints and slipped tendons.

4. Excess molybdenum caused anemia with low hemoglobin and low red blood cell counts, but did not affect the blood volume of rabbits.

5. Dietary copper was effective in preventing molybdenum toxicity and in curing all symptoms of the toxicity except the front leg abnormality.

6. Molybdenum tended to increase the accumulation of copper in the liver of experimental rabbits.

7. Molybdenum fed at the level of 0.014 and 0.02 per cent in a simplified diet retarded the growth of male rats, but did not significantly restrict the growth of female rats.

8. Vitamin A and vitamin E did not improve the growth of male rats fed 0.014 and 0.02 per cent molybdenum.

9. Molybdenum did not affect the reproduction of rats under conditions of this experiment.

10. Alopecia which was confined to thinning of the hair over the back was observed in some male rats receiving dietary molybdenum.

11. There was an increase in urinary and fecal excretion of orally administered radioactive phosphorus^{32} in rabbits receiving 0.1% molybdenum in the ration.

12. Molybdenum fed at a level of 0.01% and 0.02 per cent in the diet of rats increased the urinary excretion of phosphorus^{32} but did not increase fecal excretion.

13. The distribution of phosphorus^{32} and the amount of total phosphorus in the tissues of rabbits and rats was not significantly affected by the presence of molybdenum in the ration.

Recommendations for Future Research

The results of this research have been presented and discussed with relation to results of similar research with other species. In addition to the results which have been recorded, other observations have been made which imply still other actions of molybdenum. These proposals, if submitted to properly designed research, should provide additional approaches to the study of the role of molybdenum in the animal organism. Some recommendations for specific research in this field follow.

1. Differences in species tolerance and differences in the manifestation of molybdenum toxicity have been established for several species. These different responses to molybdenum should be more specifically related to the physiology of the animal. An example of the variations in species response is the occurrence of severe diarrhea in some species and absence of

such a symptom in others when molybdenum is in excess.

2. Further studies with rabbits should employ simplified or purified diets so that the intake of copper may be limited to a smaller amount.

3. The abnormal development of front legs in young rabbits suggested that molybdenum may create a manganese deficiency or may interfere with the metabolism of manganese. This bone abnormality should be studied with relation to manganese or to other physiological functions concerned with bone formation.

4. A comparison of the growth of rats in this study with previous experiments indicated that an improved nutritional status of the animal increases the tolerance to molybdenum. The effects of molybdenum should be further related to nutritional status of the animal.

5. The more adverse effects of molybdenum upon young animals indicated an action of molybdenum associated with the growth process.

6. The anemia with low red blood cell counts in rabbits suggested that cobalt or vitamin B_{12} might be deficient. It should be determined whether the anemia will respond to vitamin B_{12} therapy.

7. The type of anemia which develops in the different species should be more specifically identified.

8. The apparent normal libido in male rabbits suffering from molybdenum toxicity suggested that the effect upon reproduction

in rabbits may not parallel the effects observed in other species.

9. Factors responsible for the greater toxicity of molybdenum when fed with green succulent feed could be studied on a laboratory scale using the rabbit as a laboratory animal.

10. Phosphorus metabolism studies indicated that molybdenum interfered with absorption of phosphorus. Additional balance studies made at a time when animals show symptoms of molybdenum toxicity should establish more clearly the effect of molybdenum upon phosphorus absorption and excretion.

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BIOGRAPHICAL ITEMS

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While in residence at Virginia Polytechnic Institute he held a teaching fellowship. During World War II he served four years as a commissioned officer in the United States Army. In September 1946 he was employed as Assistant Professor in the Department of Dairy Science at the University of Florida. He held this position until entering graduate school at the University of Florida in February 1950 to begin studies leading toward the Doctor of Philosophy degree. While in residence for this degree he held a grant-in-aid fellowship from the Nutrition Foundation, Inc.

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This dissertation was prepared under the direction of the Chairman of the candidate's Supervisory Committee and has been approved by all members of the Committee. It was submitted to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Date June 9, 1952

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